ANNUAL PROGRESS REPORT

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EFFECTS OF DEPLETION OF SOME PHYSIOLOGICAL SUBSTANCES ON THE DEVELOPMENT OF ACUTE RADIATION SICKNESS

U.S. ARMY CONTRACT NO. DA-49-193-MD-2191

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Abstract

A preliminary study has been made of the effects of whole-body exposure to penetrating ionising radiations (X-rays, 400 rads) on blood and tissue levels of ascorbic, dehydro-ascorbic and diketo-gulonic acid. This was undertaken to test the hypothesis that the rapid oxidation of ascorbic acid to its dehydro-ascorbic form may be responsible for radiobiological damage.

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PROGRESS REPORT

ORIGINAL RESEARCH TITLE: The Effectiveness of Reserpine and of Some Substituted Indole-Alkylamines as Radioprotective Agents.

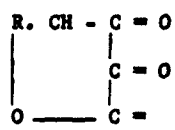
AMENDED TITLE: Effects of Depletion of Some Physiological Substances on the Development of Acute Radiation Sickness.

The original title of the contract was "The Effectiveness of Reserpine and Some Substituted Indole-alkylamines as Radioprotective Agents" subsequently amended to "Effects of Depletion of Some Physiological Substances on the Development of Acute Radiation Sickness", owing to the fact that previous experience at Walter Reed Army Research Center had shown that the first mentioned compounds held little promise as effective radioprotectants in man. Hence the decision to explore a more fundamental problem dealing with the possible role of ascorbic acid in the causation of radiobiological damage.

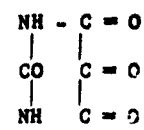
Ascorbic acid, in its reduced form, plays an important role as an antioxidising agent in blood and tissues.

Exposure of tissues to penetrating ionising radiation can hasten the slow conversion of ascorbic to dehydro-ascorbic acid, a substance capable of inducing severe cellular damage.

It is well known that while the mammalian organism will tolerate the exogenous administration of enormous doses of ascorbic acid without ill effect, small amounts of dehydro-ascorbic acid will cause severe toxic symptoms such as dyspnea, pulmonary edema, and parenchymatous damage to the liver, kidney, and pancreas. The symptomatology and site of the lesion bear close resemblance to alloxan poisoning. There are, in fact, considerable structural similarities between dehydro-ascorbic acid and alloxan.



Dehydro-ascorbic acid



Alloxan

Dehydro-ascorbic acid unlike alloxan, however, is a physiological substance which can be readily mobilised from body stores ubiquitously present in the mammalian organism.

The presence of glutathione and cysteine both in blood and tissues controls the rate of oxidation of ascorbic acid to its dehydro form and protects tissues against the toxic potentialities of the latter.

When the mammalian organism is exposed to penetrating ionising radiations, however, a relevant quota of the available -SH groups is oxidized to S-S and thus become unable to prevent the rapid oxidation of ascorbic acid into dehydro-ascorbic which will gain access to circulation and manifest its noxious effects.

The following preliminary experiment was therefore performed in order to test this hypothesis.

Young adult hybrid guinea pigs of both sexes, averaging 500 gm in weight, were obtained from a commercial breeder in Napa (California). They were housed individually in wire mesh cages and kept under observation for two weeks before being admitted into the experiment.

They received Purina Rabbit Checkers ad libitum with an addition of a measured amount of greens (50 gm, only carrots or lettuce leaves) which provided adequate Vitamin C supplementation. The animals were weighed, numbered, and randomized one week before the initiation of the experiment.

A previous determination (conducted under exactly comparable conditions on 36 animals of both sexes obtained from the same source) showed their $LD_{50/30}$ to be approximately 380 rads for whole-body exposure. This dose was therefore chosen to investigate the effect of acute whole-body exposure on ascorbic acid metabolism.

The experiment was carried out as follows: 36 guinea pigs (16 males and 16 females) whose average weight was 470 gm $\pm$ 42 gm were divided into 6 experimental groups, each comprising 6 animals (3 were sham-irradiated and 3 received 400 rads). The radiological factors of the exposure were as follows: 250-Kvcp X-rays, HVL 1.6 mm Cu, TSD = 98 cm, exposure-dose rate 44 r/minute. During irradiation the animals were restrained individually in the stalls of an exposure box having a depth of 10 cm. The path of the X-ray beam was from the dorsal to the ventral surface. From the depth-dose data it was estimated that the maximum and minimum tissue dose delivered along the path of the beam might vary by 15% and that the average tissue-dose rate was about 95% of the entrance dose. The weighted average tissue dose was equal to 92% of the reading of a Victoreen thimble chamber exposed in a stall of the exposure box, the remaining stalls contained phantoms.

The control animals were boxed under the same conditions as the irradiated ones and kept in the exposure chamber for an equivalent period of time. A group of 6 animals (3 controls and 3 exposed) were sacrificed on days 1, 2, 3, 4, 5, and 6 after irradiation.

Ascorbic, dehydro-ascorbic, and diketo-gulonic acid were determined in the blood, kidney, liver, and spleen of the control and irradiated animals with the differential ascorbic acid method of Roe, Mills, Oesterline and Damron. In order to prevent variations in blood and tissue levels of ascorbic acid due to irregular food intake in the irradiated animals, careful pair-feeding with the controls was instituted.

The results showed some fluctuations in the blood and tissue levels of the three constituents measured, as shown in the attached table.

TABLE 1

	Ascorbic Acid		Dehydro-ascorbic Acid (in mg/100 gm of wet tissue)		Diketo-gulonic Acid	
	Exposed	Control	Exposed	Control	Exposed	Control
Liver	10.25	9.49	.751	.741	.433	.508
Kidney	5.02	5.06	.585	.741	.863	.718
Spleen	76.31	90.06	34.77	38.33	22.80	26.63
Blood	.208	.150	.286	.278	.032	.052

Mean tissue and blood levels of ascorbic, dehydro-ascorbic, and diketo-gulonic acid determined on each of the six post-irradiation days. The analysis of variance performed for each organ of the control and experimental group failed to show any significant change.

The inability to detect a definite change in the variables under study may be due to the relatively low dose of radiation employed or to the choice of an animal which is unable to synthesize ascorbic acid, and is likely to show wide fluctuations in its blood and tissue levels of ascorbic acid and its congeners. The possibility that changes take place only during exposure or in the hours which immediately follow exposure will also be considered, and this hypothesis will be tested in a future experiment.

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There are, however, good indications that ascorbic acid metabolism is involved in the mechanism of radiation damage: a recent report (N. Y. Glushakova, F. M. Laguto and O. S. Luchenok: Radiation Sickness and Vitamin C in the Liver and the Wall of the Small Intestine of Animals which Synthesized Ascorbic Acid - Meditsinskaya Radiologyia, Vol. V, No. 4, Moscow 1960) shows that the ability of the liver of irradiated rats to reduce dehydro-ascorbic acid is depressed after exposure to 700 r. This occurs particularly in the immediate post-irradiation period.

In a forthcoming series of experiments with rats, we are planning to repeat our experiment with two doses of whole-body X-ray $LD_{50/30}$ and the $LD_{100/30}$ (400 and 800 rads) and to determine the activity of dehydro-ascorbic reductase in the liver immediately following exposure.

A series of $LD_{50/30}$ determinations will also be performed in rats depleted of ascorbic acid by previous treatment with chlorobutanol and in rats to which scalar doses of ascorbic or dehydro-ascorbic acid have been administered shortly before exposure.

The influence of these procedures on mortality will be studied.

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